Analysis of Anabolic Steroids

1. Background

Steroids come in two forms which are anabolic and androgenic. Anabolic steroids promote muscle growth, enhance athletic or other physical performance and improve physical appearance. Androgenic steroids promote the development of sex characteristics and maintain traits such as body hair, deepening of the voice and baldness. Regardless of their specific physiological role, all steroids contain the same basic cyclopentanoperhydrophenanthrene ringed nucleus. The variation on the basic nucleus determines the specific steroid and its properties. Steroids are most commonly found as injectable oils, tablets and capsules. Other forms may include powders, creams, liquid drops and transdermal patches.

2. Objective

The objective of this SOP is to establish guidelines to be used for the analysis of a sample that may contain an anabolic steroid.

3. Scope

This SOP is to be used by the laboratory staff of the Division of Analytical Chemistry at William A. Hinton State Laboratory Institute in Boston, MA.

4. Responsibility

Chemists are responsible for acquiring glassware, preparing chemical reagents and standards, sample analysis, and reporting. Chemists also perform instrument calibrations, maintenance and troubleshooting, ordering of supplies and other necessary tasks related to this analysis.

Technical Reviewers will review each case and complete the comprehensive reviewer checklist. They will ensure that the chemist followed this SOP. The Technical Reviewer may perform the duties and responsibilities of the chemist.

Laboratory Supervisors ensure that chemists are following this SOP. They may perform the duties of the chemists and must review raw data and reports generated by chemists. The Supervisor may advise the chemists of alternative testing methods. They ensure that quality control measures are within acceptable limits and determine when corrective actions are needed. They coordinate proficiency testing (PT), reporting and distribution of PT results. They oversee sample results distribution to outside agencies.

Directors ensure that the SOP is being followed and reviewed on a regular basis. They provide approval of standard operating procedures and review quality control documentations.

1. Related Documents

Cole, Michael, "The Analysis of Controlled Substances," London: John Wiley & Sons Ltd., 2003
Drug Enforcement Administration, "Basic Training Program for Forensic Drug Chemists," Drug Enforcement Administration.
Mills III, Terry et al, "Instrumental Data for Drug Analysis," 3rd ed., 6 vols., New York: CRC Press, 2006.
Moffat, A.C. et al, "Clarke's Isolation and Identification of Drugs," 2nd ed., London: The Pharmaceutical Press, 1986.
Moffat, A.C. et al. "Clarke's Analysis of Drugs and Poisons," 3rd ed., London: The Pharmaceutical Press, 2004.
Saferstein, Richard, "Forensic Science Handbook," New Jersey: Prentice Hall, 1988.
Scientific Working Group for the Analysis of Seized Drug Recommendation, 6th ed., "Part III A & B, Methods of Analysis/Sampling of Seized Drug for Qualitative Analysis," July 2011

6. Definitions

GC w/ FID: Gas Chromatography with Flame Ionization Detector
GC/MS: Gas Chromatography/Mass Spectrometry
Gross Weight: The weight of both the substance and its container.
Net Weight: The weight of the substance only.

7. Supplies, Equipment & Reagents

Supplies

Culture tubes (disposable, glass, 12 x 75mm or equivalent)
GC columns
HP-1MS (Agilent, Cat # 19091S-933UI or equivalent)
HP-5MS (Agilent, Cat # 19091S-433UI or equivalent)

GC crimp vials
- Clear (Agilent, 2mL, Cat # 5182-0543 or equivalent)
- Amber (Agilent, 2mL, Cat # 5181-3376 or equivalent)

Pasteur pipette
Pestle
Porcelain spot plate
Scapula
Scissors
Spatula
Stirring rod
Syringes with needle (Terumo, Safeguard2, 3cc with 23G x 1 or equivalent)
Teflon crimp (top) caps
- Silver (Agilent, Cat # 5181-1210 or equivalent)
- Blue (Agilent, Cat # 5181-1215 or equivalent)

Various Class A glassware
- Beakers
- Graduated cylinders
- Volumetric flask

Weighing dish (VWR, Anti-Static, Cat # 89106 or equivalent)
Weighing paper (VWR, Cat # 12578 or equivalent)

Equipment

Analytical Balance (range 0.0001g to 1.0g)
GC with FID (Agilent, Model # 7890 Series or equivalent)
GC/MS (Agilent, Model # 5975 Series or equivalent)

Reagents

Acetone (JT Baker, Ultra Resi-Anhydrous, Cat # 9254 or equivalent)
Chloroform (JT Baker, ACS Grade, Cat # 9180 or equivalent)
Cobalt thiocyanate (Aldrich, Cat # 216135 or equivalent)
Cobaltous acetate tetrahydrate (Fisher Scientific, Certified, Cat # C364 or equivalent)
Deionized water (in-house)
Formaldehyde, 37% wt solution (Acros Organic, ACS Grade, Cat # AC41073 or equivalent)
Glacial acetic acid (JT Baker, ACS Grade, Cat# 9511 or equivalent)
Hydrochloric acid (JT Baker, ACS Grade, Cat # 9535 or equivalent)
Isoproplyamine (Acros Organic, 99%, Cat # AC14892 or equivalent)
Methanol (JT Baker, ACS Grade, Cat # 9070 or equivalent)
Selenous acid (Acros Organic, 99.999%, Cat # 43712 or equivalent)
Sodium molybdate (JT Baker, ACS Grade, Cat # 3764 or equivalent)
Sulfuric acid (JT Baker, ACS Grade, Cat # 9681 or equivalent)

Standards

17α-Methyltestosterone
17α -Estradiol
1-Dehydrotestosterone
Anastrozole
Androstenediol

Bolandiol
Boldenone Undecylenate
Cocaine Hydrochloride
Codeine Phosphate
Drostanolone
Drostanolone Propionate
Estrone
Mesterolone
Methandrostenolone
Methenolone
Methyldihydrotestosterone
Nandrolone
Nandrolone Decanoate
Nandrolone Phenpropionate
Norgestrel
Ondansetron Hydrochloride Dihydrate
Oxandrolone
Oxymethalone
Stanozolol
Testosterone
Testosterone Decanoate
Testosterone Enanthate
Testosterone Phenylpropionate
Testosterone Propionate
Trenbolone Acetate
Trenbolone Enanthate

8. Safety

Due to the potential hazards, appropriate precautions should be taken as necessary. This includes, but is not limited to, the use of fume hoods, gloves, masks and safety glasses. Lab coats are to be worn at all times in the unit, unless performing administrative duties.

9. Reagent Preparation

Anastrozole Standard
Dissolve 2.0mg of anastrozole and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Androstenediol Standard
Dissolve 2.0mg of androstenediol and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Boldenone Undecylenate Standard
Dissolve 4.0mg of boldenone undecylenate and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Bolandiol Standard

Dissolve 0.2mg of bolandiol and bring to volume with 0.2mL of methanol. Mix the solution until completely dissolved.

1-Dehydrotestosterone Standard
Dissolve 2.0mg of 1-dehydrotestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Drostanolone Standard
Dissolve 2.0mg of drostanolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Drostanolone Propionate Standard
Dissolve 2.0mg of drostanolone propionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Estrone Standard
Dissolve 2.0mg of estrone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

17α -Estradiol Standard
Dissolve 2.0mg of 17α–estradiol and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Mesterolone Standard
Dissolve 2.0mg of mesterolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Methandrostenolone Standard
Dissolve 2.0mg of methandrostenolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Methenolone Standard
Dissolve 0.2mg of methenolone and bring to volume with 0.2mL of methanol. Mix the solution until completely dissolved.

Methyldihydrotestosterone Standard
Dissolve 1.0mg of methyldihydrotestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

17α-Methyltestosterone Standard
Dissolve 2.0mg of 17α-methyltestosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Standard
Dissolve 2.0mg of nandrolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Decanoate Standard
Dissolve 2.0mg of nandrolone decanoate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Nandrolone Phenpropionate Standard
Dissolve 2.0mg of nandrolone phenpropionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Norgestrel Standard
Dissolve 2.0mg of norgestrel and bring to volume with 1.8mL of acetone. Mix the solution until completely dissolved.

Ondansetron Hydrochloride Dihydrate Standard
Dissolve 2.0mg of ondansetron hydrochloride dihydrate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Oxandrolone Standard
Dissolve 2.0mg of oxandrolone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Oxymethalone Standard
Dissolve 1.0mg of oxymetholone and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Stanozolol Standard
Dissolve 4.0mg of stanozolol and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Testosterone Standard
Dissolve 2.0mg of testosterone and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Decanoate Standard
Dissolve 2.0mg of testosterone decanoate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Enanthate Standard
Dissolved 2.0mg of testosterone enanthate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Phenylpropionate Standard
Dissolve 2.0mg of testosterone phenylpropionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Testosterone Propionate Standard
Dissolve 2.0mg of testosterone propionate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Trenbolone Acetate Standard
Dissolve 2.0mg of trenbolone acetate and bring to volume with 1.8mL of chloroform. Mix the solution until completely dissolved.

Trenbolone Enanthate Standard
Dissolve 2.0mg of trenbolone enanthate and bring to volume with 1.8mL of methanol. Mix the solution until completely dissolved.

Cobalt Thiocyanate Reagent
Dissolve 2.0g of cobalt thiocyanate in 100mL of deionized water. Mix the solution until completely dissolved.

Marquis Reagent
Dilute 10mL of 37% formaldehyde solution in 90mL of concentrated sulfuric acid. While stirring, slowly add the concentrated sulfuric acid to the formaldehyde solution. Allow the solution to cool completely.

Froedhde's Reagent
Dissolve 0.5g of sodium molybdate in 100mL of concentrated sulfuric acid. Mix the solution until completely dissolved.

Mecke's Reagent
Dissolve 1.0g of selenous acid in 100mL of concentrated sulfuric acid. Mix the solution until completely dissolved.

Dilly Koppanyi Reagent
Dilly
Dissolve 0.1g of cobaltous acetate tetrahydrate in 100mL of methanol. Then add 0.2mL of glacial acetic acid to the solution. Mix the solution until completely dissolved.
Isopropylamine
Dilute 5mL of isoproplyamine in 100mL of methanol. Mix the solution completely.

2.8N Hydrochloric Acid Reagent
Dilute 92.6mL of 12.1N hydrochloric acid in 400mL of deionized water. Mix the solution completely.

20% Acetic Acid Reagent
Dilute 100mL of glacial acetic acid in 400mL of deionized water. Mix the solution completely.

Cocaine/Codeine Standard (QC Mix)
Dissolve 10.0 mg of cocaine hydrochloride and 10.0mg of codeine phosphate and bring to volume with 10mL of methanol. Mix the solution until completely dissolved.

10. Procedure

A. Evidence Handling

i. Evidence Officer will randomly assign sample to a chemist.

ii. The chemist will perform an evidentiary check on the sample. They will verify that the manila envelop, control card and the evidence correspond. They will observe the integrity evidence bag and its contents.
iii. Once the sample has being verified, the chemist will take custody of the samples by signing out the evidence in the chain of custody logbook.
iv. The sample will be brought to the chemist work area and stored in a secure manner at all times.
v. Upon analysis of each sample, the chemist will document all observations on the Drug Analysis Form.
vi. The information on the Drug Analysis Form will contain but not limited to the sample number, submitting agency, verification of the evidence gross weight, number of samples, container, type of sample, color, viscosity, gross and net weight, ballistics of sample, chemist notations and results, preliminary and confirmatory findings.
vii. Obtain the weight of the sample.
    a. For liquid, cream/gel and residual patches, only a gross weight should be documented.
    b. For powders and tablets, both a gross weight and net weight should be documented.
viii. Androgenic steroids or human growth hormones should not be analyzed and will be documented as Not Tested.

B. Sampling Plan

i. (to be determine)

C. Sample Preparation

i. For powders, no preparation is needed for color test. For GC or GC/MS analysis, place 1-2 mg of sample into a labeled test tube and dissolve with 1-2mL of methanol. Vortex and let stand for ½ hr.
ii. For tablets, use ¼ of the tablet and crush into a powdered form. For GC or GC/MS analysis, place 1-2mg of the sample into a labeled test tube and dissolve with 1-2mL of methanol. Vortex and let stand for ½ hr.
iii. For liquids, extract ½ mL of the liquid using a syringe and place into a labeled test tube. For GC or GC/MS analysis, place 2-4 drops of the sample into a labeled test tube and dilute with 1-2 mL of methanol. Vortex and let stand for ½ hr.
iv. For gels or creams, remove a 3-4mg amount of sample and place into a labeled test tube. For GC or GC/MS analysis, place 1-2mg of the sample into a labeled test tube and dissolve with 1-2 mL of methanol. Vortex and let stand for ½ hr.
v. For transdermal patches, cut ½ of the patch into smaller pieces and place into a labeled test tube. For GC or GC/MS analysis, place ½ of the cut pieces of the sample into a labeled test tube and dissolve with ½ - 1mL of methanol. Vortex and let stand for ½ hr.

D. Color Tests

i. The color test consists of five reagents, which are cobalt thiocyanate, marquis, froehde's, mecke's and dilly koppanyi.
ii. For powdered substances, place a couple of drops of cobalt thiocyanate, marquis, froehde's, mecke's reagents into four individual wells on a porcelain spot plate. Then add a small amount of sample (1-2mg of powder) to each well. Note any color change or reaction.
iii. For liquid substances, add a small amount of sample (1-2 drops) into four individual wells on a porcelain spot plate and allow the sample to dry completely. Then place a couple of

drops of cobalt thiocyanate, marquis, froehde's, mecke's reagents into each wells. Note any color change or reaction.

iv. For dilly koppanyi color test, add a small amount of sample (1-2mg of powder/gel or 1-2 drops of liquid) to a labeled test tube. Then add equal amounts (1ml) of dilly and isopropylamine reagents respectively to the test tube. Note any color change or reaction.

v. The results will be recorded on the Drug Analysis Form by documenting the actual color/s observed. Negative observations will be recorded by stating no reaction or no color change.

E. Gas Chromatography Screen

i. The methanolic extract from section (C) can be used for the GC analysis. Pipette the extract into a labeled GC vial and cap tightly.

ii. Initiate auto sampler sequence using the GENSCAN method running a blank solvent between each unknown sample and reference standard/s.

iii. Compare retention time of the each sample with the reference standard/s. Also check the chromatograph to determine if the sample needs to be diluted or concentrated.

iv. Positive GC analysis will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when the retention time of the sample and the reference standard meet the laboratory criteria and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

F. Gas Chromatography/Mass Spectrometry

i. Confirmatory analysis can be performed using the labeled GC vial from the previous section (E).

ii. Initiate auto sampler sequence using the SCREEN method running a blank solvent between each unknown sample.

iii. Using the chromatogram and ion spectra, determine whether or not an illicit narcotic is present.

iv. Document the results on the MS Tracking Sheet. For positive GC/MS, document which illicit narcotic is present and its concentration. For negative results, document observations by stating negative on the results section.

v. If an illicit narcotic is present, choose the appropriate solvent for the sample, if needed.

vi. Initiate auto sampler sequence using the appropriate method running a blank solvent between each unknown sample and the reference standard/s.

vii. Compare retention time and ion spectra of the each sample with the reference standard/s.

viii. Document the date analyzed and results of the GC/MS onto the MS Tracking Sheet, Drug Analysis Form and Control Card.

G. Criteria for Gas Chromatography/Mass Spectrometry

i. Retention time of the sample must be within +/- 1.5% of the reference standard.

ii. Library spectra match must be > 90%.

iii. There must be a visual spectral match between the reference standard and the sample.

iv. At least 5 of the major ions must be present for the sample.

11. Documentation

A. All results will be documented on the Drug Analysis Form.

B. All raw data will be generated and filed according to the laboratory policy.

C. A certificate of analysis will be generated for each lab number which will document the results.

12. Attachments